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14. ABSTRACT

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It is becoming increasingly evident that improving the cure rate of many cancers will require treatment regimens hit more than one validated tumor targets. Developing an anti-cancer agent that targets two oncoproteins simultaneously is a promising strategy for accomplishing this goal. It would be expected to promote drug efficacy, reduce therapy-resistant without introducing additional toxic side effects. Recently, we have identified TEL03 from Chinese medicinal herb significantly inhibits activations of HIF-1α/2α and phosphorylated Stat3 (p-Stat3), and blocks the expression of their down-regulated oncogenes (e.g. Bcl2, VEGF, Glut1, and others) in cancer cells. TEL03 also dramatically suppressed the growth of prostate, breast and pancreatic tumors in xenograft models. Our previous studies provided evidence that targeting both HIF-1α and Stat3 together could improve tumor response to either agent alone, and reduce drug resistance and treatment failure. TEL03 was showed inhibition of tumor growth and a marked delay in tumor re-growth, and demonstrated a greater-than-expected in vivo potency. Our results also provided evidence that TEL03 has potential to be a potent anti-cancer agent for pancreatic cancer therapy, suggesting that TEL03 could have a possible clinical application in prostate cancer therapy.

15. SUBJECT TERMS
Stat3, HIF-1alpha, TEL03, cancer therapy, Prostate cancer.

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Table of Contents

Page

ANNUAL REPORT FOR PC093258 (W81XWH-09-PCRP-IDA)

PI: Naijie Jing

A. INTRODUCTION

A1. Title: Developing a novel agent to target HIF-1α and Stat3 is important for prostate cancer therapy

A2. Significance

Prostate cancer is the most common cancer in men worldwide, accounting for approximately 242,000 new cases and 28,000 deaths annually in the USA. Although localized disease is often curable, advanced disease is generally not, especially when the cancer becomes castration resistant (mCRPC) and metastasizes. Docetaxel-based cytotoxic chemotherapy is most common therapy for mCRPC (1). Most prostate cancer (PC) patients are treatable, but the patients usually die due to drug resistance and metastatic disease (2). Hypoxia is one of the fundamental biological phenomena that are intricately associated with the development and aggressiveness of a variety of solid tumors including prostate cancer. Tumour hypoxia is progressively emerging as a common feature of prostate tumours associated with poor prognosis (3). HIF-1 is the only DNA regulatory element truly regulated by oxygen. Cross-talk between the AR and HIF-1α in prostate cancer cells has recently been identified (4).

HIF-1 protein is a heterodimer consisting of two subunits: HIF-1α and HIF-1β. Under normoxia, prolyl hydroxylases (PHDs) hydroxylate the prolyl residues of HIF-1α at amino acids P402 and P564, which are then recognized by VHL (Von Hippel-Lindau) and targeted to the ubiquitin proteasome pathway. An additional hydroxylation at N803 blocks the binding of p300 and CBP (Creb-binding protein) to HIF-1α and inhibits HIF-1-mediated gene transcription. Under hypoxia, HIF-1α is not hydroxylated and not degraded. The unmodified protein then dimerizes with HIF-1β. As N803 is not hydroxylated, p300 (or CBP) can bind to HIF-1α, allowing transcriptional activation of HIF-1 target genes, which are included more than 70 putative hypoxia-inducible genes, to date, and involved in many cell processes including glucose metabolism, erythropoiesis, angiogenesis, anti-apoptosis, metastasis, and other functions (5-10). HIF-1α was demonstrated to overexpress in many human cancers, including prostate cancer (11-13). Overexpression of HIF-1α not only strongly enhances the tumor growth rate and metastatic potential, but also contributes to resistance to radiotherapy and chemotherapy, leading to treatment failure and increase in patient mortality (14, 15).

Stat3 (signal transducer and activator of transcription 3) exist as monomers or N-terminal head-to-head dimers in the cytoplasm (16,17). When stimulated by cytokines or growth factors, such as JAK, Scr or EGFR (18, 19), Stat3 is activated upon phosphorylation on tyrosine residue Y705 (20). Tyrosine phosphorylation induces formation of a parallel dimer through their SH2 domains (18, 20). The activated dimers translocate to the nucleus, where they bind to DNA-response elements in the promoters of target genes and activates transcription. Stat3 participates in oncogenesis through the upregulation of genes encoding anti-apoptosis (Bcl-x_L, Bcl-2, Mcl-1, and survivin), cell-cycle regulators (cyclin D1 and c-*myc*), and inducers of angiogenesis (VEGF) (14, 24, 25). Also, immune system plays a crucial role in controlling tumor incidence and growth. Stat3 signaling is a major intrinsic pathway of cancer inflammation and mediates the cancer-promoting properties. Stat3 suppresses anti-tumor immune responses and promotes inflammation-induced cancer, making it an attractive target (25-27).

A3. Innovation

1. TEL03 is a novel anti-cancer agent from Chinese herbal medicine (CHM) that targets HIF-1α/2α and Stat3. Recently, we have identified a perylene derivative TEL03 from *hypocrellin* (CHM: *Hypocrella bambusae*) targets HIF-1α/2α and Stat3, significantly inhibits activations of HIF-1α/2α and phosphorylated Stat3 (p-Stat3), and blocks the expression of their down-regulated oncogenes (e.g. Bcl2, VEGF, Glut1, and others) in cancer cells. TEL03 also can significantly suppress the growth of prostate and pancreatic tumors in xenograft models. Our previous studies (28) provided evidence that

targeting both HIF-1α and Stat3 together could improve tumor response to either agent alone, and reduce drug resistance and treatment failure. Stat3 (signal transducer and activator of transcription 3) participates in oncogenesis through the upregulation of genes encoding anti-apoptosis, cell-cycle regulators, and inducers of angiogenesis in many cancers, inclosing breast cancer (29-31). Also, immune system plays a crucial role in controlling tumor incidence and growth. Stat3 signaling is a major intrinsic pathway of cancer inflammation and mediates the cancer-promoting properties. Stat3 suppresses anti-tumor immune responses and promotes inflammation-induced cancer, making it as a critical target for cancer therapy (32,33). Our results showed that TEL03 as a dual inhibitor can promote drug efficacy, reduce emergence of therapy-resistant cancer cells, and decrease the incidence of cancer relapse with a low toxicity.

2. Extracting new anti-cancer compounds from CHM is a pioneering strategy. Although anti-cancer agents have developed quickly, new anti-cancer agents are still urgently required. The advantages of TEL03 provide evidence that it is a pioneer strategy to develop a promising anti-cancer agent from new compounds that are extracted from CHM. CHM is a major aspect of traditional medicine, which has been developed as part of Chinese culture since 200 BC. CHM, which is mainly obtained from nature plants (~95%), focuses on restoring a balance of energy, body, and spirit to maintain health rather than treating a particular diseases or medical condition. Also, they can help ease the side effects of standard cancer treatment, control pain, improve quality of life, strengthen the immune system, and in some cases, stop tumor growth and spread. With collaborating with Dr. Yuan group in Peking University (China), we have extracted many compounds from CHM, which mostly are new compounds without previously studied, and discovered few compounds that activity against the molecular target: HIF-1α, such as a perylene derivative TEL03 derived from *hypecrellin* and NDT extracted from roots of *Zanthoxylum nitidum* (see below). Therefore, we propose to develop potent anti-cancer agents from CHM that targets HIF-1α for prostate cancer therapy.

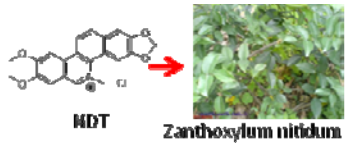

Fig.B1. NDT extracted from Z.nitidum

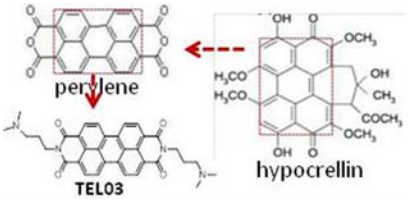

Fig.B2a. TEL03 is a derivative of perylence, which is origenal derived from hypocrellin.

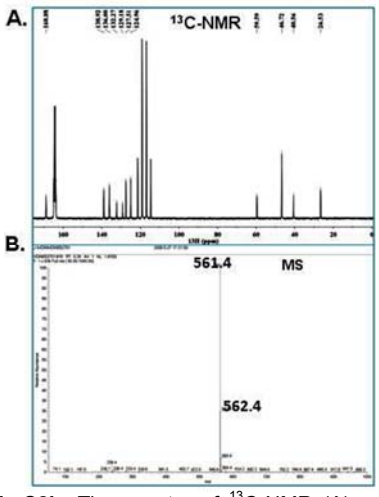

Fig.C2b. The spectra of ^{13}C-NMR (A) and MS (B) for the synthesized TEL03.

B. KEY RESEARCH ACCOMPLISHMENTS

B1. Extracting chemical compounds from CHM. In order to develop novel anti-cancer agents, we have extracted many compounds from CHM,I such as: *Nitidine chloride (NDT) from Roots of Zanthoxylum nitidum (Fig.B1).* The air-dried powdered roots of Z. nitidum (1Kg) were extracted with ethanol (95%, v/v, 3×5L) under reflux for 3×3h. The ethanol extract (15L) was subjected to cation exchange resins CC (gradient saturated sodium hydroxide in 60-90% ethanol) to afford an eluent contained nitidine chloride depending on the TLC behavior comparison with authentic sample. The solution contained nitidine chloride was concentrated under reduced pressure with a rotary evaporator to produce a total alkaloids deposit. The solution contained nitidine chloride was concentrated under reduced pressure with a rotary evaporator to produce a total alkaloids deposit.

B2. Drug synthesis based on the derivatives from *hypocrellin*. TEL03 as a lead compound was chemical synthesized based on perylence, which was derived from *hypocrellin* (Fig.B2a).

The method of synthesis The TEL03 was synthesized by the reaction that 0.2g (0.51mmol) of 3,4,9,10-perylenetetracarboxylic dianhydride was dissolved in 30mL n-Butanol heating to 90 °C and 0.8mL (6.3mmol) of 3-(dimethylamino)propylamine was added to the mixture, then kept the temperature for 4h. Cooling the mixture to

room temperature, the precipitation was collected, and washed by chloroform and methanol. The molecular structure and purification of TEL03 were identified by H^1-NMR, C^{13}-NMR and Mass Spectrum (Fig.B2b). The HPLC results also showed that the purity of TEL03 is higher than 98%. Then, 0.1g (0.18mmol) TEL03 was mixed with 2mL methanol heating to 60°C, and 2mL solution of saturated hydrochloric acid in acetic ether was added to the mixture. After cooling down, the precipitation was washed by ethyl acetate.

B3. Screen for novel anti-cancer agents. The pathways: HIF-1 and Stat3 have been demonstrated to be important targets for breast cancer therapy (31). To determine whether each compound has potential to suppress the activation of HIF-1α or Stat3 in cancer cells, the assay of western blot was employed to screen all the molecules in 10μM concentration. Since our previous experiments showed if adding 10μM concentration of a compound in cell cultures, this compound still can not inhibit the targeted molecules, thus, it cannot be an active drug. After screened more then 24 compounds extracted from CHM, we found that the molecules NDT (#2) and TEL03 (#5&#12) have ability to inhibit HIF-1α expression in cancer cells under hypoxia. Meantime, TEL03 also showed the inhibition of phosphorylated Stat3 (p-Stat3) without blocking total Stat3 (T-Stat3), which is mainly composed of unphosphorylated Stat3. Thus, TEL03 can inhibit the activation of both Stat3 and HIF-1α (Fig.B3).

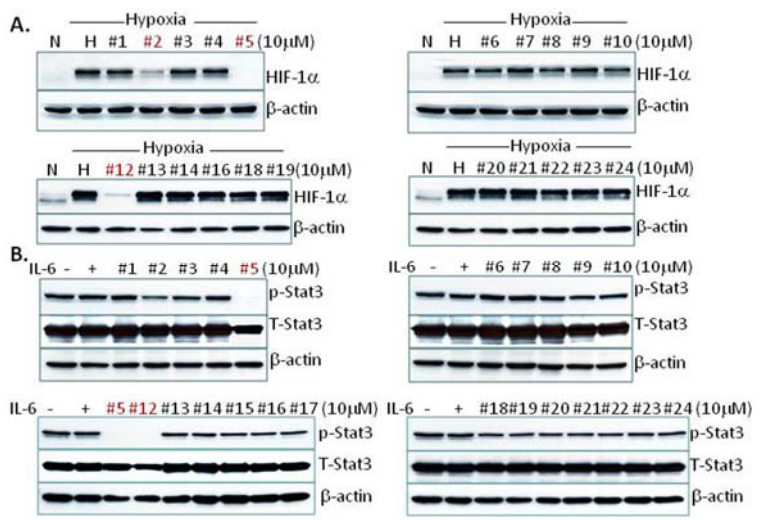

Fig.B3. Screening the compounds for HIF-1α inhibitor under hypoxia (A) and p-Stat3 inhibitor (B) in MDA-MB-231(note: N means normoxia).

B4. TEL03 selectively targets HIF-1α and Stat3. Comparing the screen data of NDT (#2) with TEL03 (#5), we selected TEL03 as a first candidate to further study. Here we performed immunoblotting assay using human cancer cells, including breast, pancreatic, ovarian and other cancer cells to confirm TEL03 drug activity and selectivity. The results showed (Fig.B4) that TEL03 significantly inhibits the expressions of both HIF-1α/2α and p-Stat3 in breast cancer cells under normoxia and hypoxia environments. The IC50s of inhibition of HIF-1α/2α and p-Stat3 for TEL03 are ~3μM in breast cancer cells. TEL03 also inhibited the expression of the down-regulated protein Bcl2 under both normoxia and hypoxia. Importantly, TEL03 did not inhibit up-stream proteins: JAK, SCR, and the most conservative proteins: p-Stat1, p-Stat5 and total Stat3 in Stat signaling, and also did not inhibit the critical proteins in HIF-1 signaling: p300 and HI-1β as well. Also we have checked off-target effects in other passways, including AKT and ERK, TEL03 did not show inhibition to p-AKT and p-ERK (Fig.B4). Therefore, TEL03 has a selective target HIF-1α and Stat3 in this range.

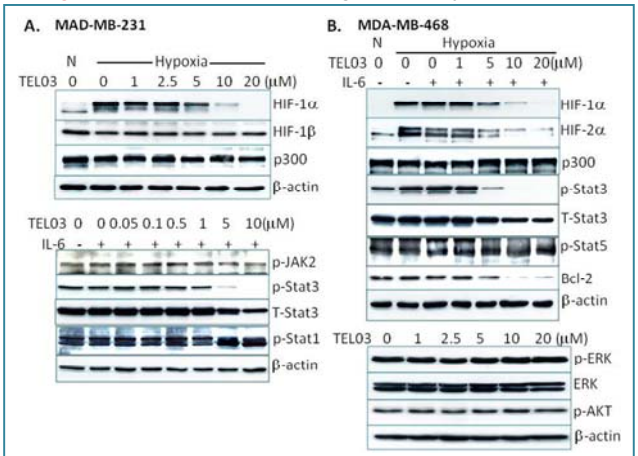

Fig.B4. TEL03 inhibits both HIF-1α/2α and Stat3 (N: normoxia, in which HIF-1α is rapidly degraded by ubiquitin-proteasome.

B5. The mechanism of TEL03 inhibiting HIF-1α and Stat3

(1)TEL03 directly targets both HIF-1α and Stat3. To identify whether TEL03 inhibits HIF-1α through the inhibition of Stat3 or TEL03 directly inhibits HIF-1α, the two assays were employed: (1) under hypoxia we used T40214 that is a developed Stat3 inhibitor (34,35) to inhibits p-Stat3 in cancer cells, and then after 3 hours added TEL03 to detect whether TEL03 can inhibit HIF-1α in the sample without p-Stat3; and (2) we performed qRT-PCR to measure RNA levels of HIF-1α since if TEL03 inhibits HIF-1α activity through the inhibition of Stat3, TEL03 should strongly suppress the RNA expression of HIF-1α. Fig.B5A showed that T40214 totally inhibited p-Stat3 activity but did not inhibit HIF-1α. HIF-1α was totally inhibited after adding TEL03, showing that TEL03 directly suppresses HIF-1α expression in the absent of p-Stat3. Also, the PCR data demonstrated (Fig.B5B) that TEL03 does not suppress the RNA level of HIF-1α in cancer cells, suggesting that TEL03 inhibits HIF-1α by targeting HIF-1α protein in hypoxic cells.

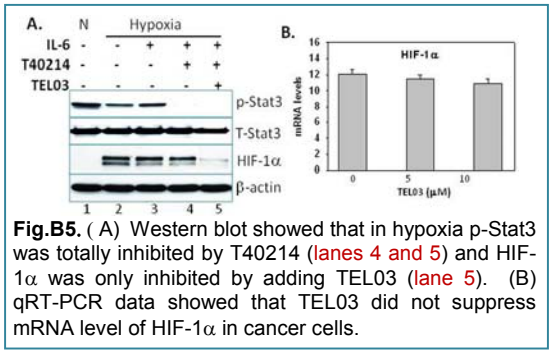

Fig.B5. (A) Western blot showed that in hypoxia p-Stat3 was totally inhibited by T40214 (lanes 4 and 5) and HIF-1α was only inhibited by adding TEL03 (lane 5). (B) qRT-PCR data showed that TEL03 did not suppress mRNA level of HIF-1α in cancer cells.

(2) The mechanism of TEL03 inhibiting HIF-1α. A GST pull-down assay was employed to determine the whether TEL03 specifically interacts with HIF-1α protein. Fig.B6A shows that p300 did not bind with GST (lane1). The samples of GST- HIF-1α/p300 and GST-HIF-1α/p300+TEL03 have an equal level of HIF-1α proteins (lanes2&3); however, the level of p300 in lane 3 is much less than that in lane 2. These results clear demonstrated that p300 strongly binds with HIF-1α in hypoxia (lane2) and TEL03 directly interacts with HIF-1α protein and blocks the binding interaction between HIF-1α and p300 (lane3).

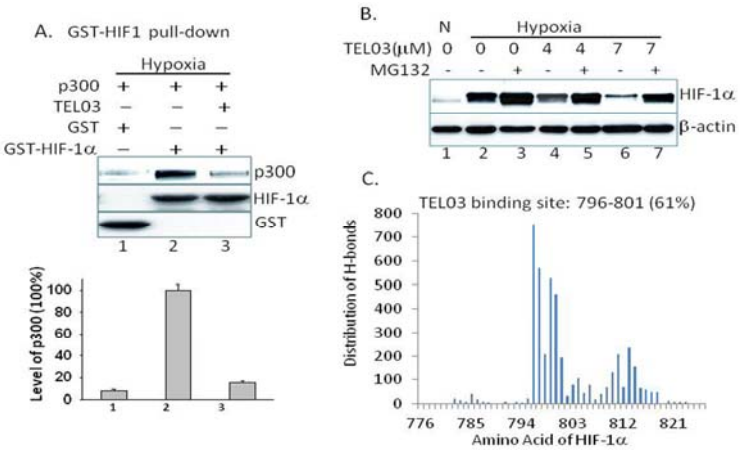

Fig.B6. (A) The pull-down data (B) The data shows that TEL03 greatly induce HIF-1α degradation through proteasome in hypoxic cells (MG132 is an inhibitor of proteasome). (C) The distribution of H-bonds that formed between TEL03 and the C-terminal domain of HIF-1α. The H-bonds were highly concentrated in the region of amino acids 796 to 801 (61%).

To determine whether TEL03 can promote the proteasomal degradation of HIF-1α in hypoxic cells, cancer cells (MDA-MB-468) were exposed to TEL03 alone or TEL03 plus MG132, which is an inhibitor of proteasome, at 1% O_2 for 18 hrs. Comparing the hypoxia-induced levels of HIF-1α with MG132 (proteasome blocked) and without MG132 (proteasome activated), the results demonstrated that TEL03 greatly induce the degradation of HIF-1α through proteasome in hypoxic cells (Fig.B6B).

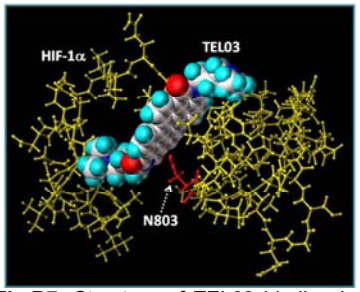

Fig.B7. Structure of TEL03 binding in the region of residues of 709 to 801 of HIF-1α in hypoxia.

To gain insight in the molecular interaction between TEL03 and HIF-1α, we randomly docked each TEL03 1000 times onto the C-terminal domain of HIF-1α (36) without setting any constraints and then analyzed the distribution of hydrogen (H) bonds formed between each TEL03 and HIF-1α because H-bonds play an important role in governing the interaction between HIF-1α and TEL03. The analysis showed that the hydrogen bonds formed between TEL03 and the C-terminal domain of HIF-1α were

highly concentrated in the region of amino acids 796 to 801 (61%) (Fig.C6C). Binding energy of TEL03 into the residues 796 to 801 in C-terminal of HIF-1α is about -52 kcal, leading to the stable binding complex of HIF-1α and TEL03 (Fig.B7). Combined all together, it suggests that TEL03 can bind in the range of residues 796 to 801 of HIF-1α to block the interaction between p300 and N803 of HIF-1α for transcription and then induce the proteasomal degradation of HIF-1α in hypoxic cells.

(3) TEL03 disrupts Stat3 phosphorylation. First, the time-dependent western showed (Fig.B8A) that TEL03 significantly inhibited phosphorylated Stat3 (p-Stat3) activity in 30 mins but did not inhibit total Stat3 (T-Stat3), JAK2, and SRC kinases within 6 hours. This result provided evidence that TEL03 blocks Stat3 phosphorylation. Then we performed SPR (surface plasmon resonance) based binding assay to determine whether TEL03 has ability to block Stat3 phosphorylation by interrupting the EGFR ligand binding to Stat3. Previous studies identified that the residues of K591, R609, S611, E612, S613, E638, and Y640 in Stat3 SH2 domain form a pocket binding with the ligand motif of EGFR (e.g. pYxxx-ligand motif) (37,38). The biotinylated pYxxx-ligand motif (pY-1068: LPVPE(pY)INQSVP) was first immobilized on a streptavidin coated sensor chip (39,40). Then adding Stat3 alone and Stat3+TEL03 complex into the chip, we observed that increaseing TEL03 reduced the binding possibility between Stat3 and the peptides, demonstrating that TEL03 interrupted the peptide receptor binding to Stat3 for phosphorylation (Fig.B8B). The modeling structure of TEL03/Stat3 complex, which was built from docking calculation using energy minimization and molecular dynamics under AMBER force fields described previously, predicted that TEL03 has potential to bind with the residues of E612, S613 and E638 in SH2 domain and disrupts the binding interaction between Stat3 and the phosphotyrosine-stimulating receptor (Fig.B8C).

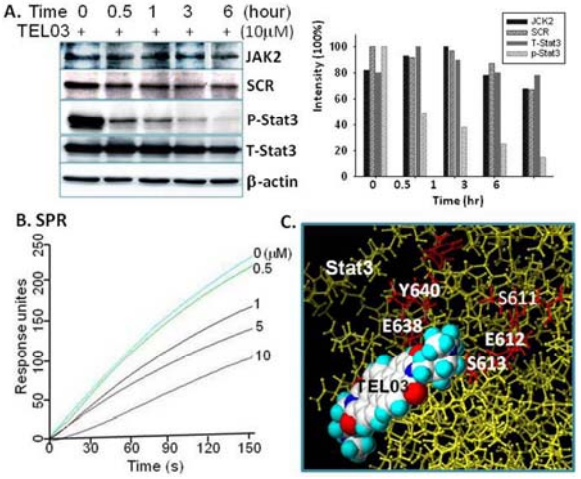

Fig.B8. (A) Time dependent western blot. (B) SPR data. (C) The molecular modeling of TEL03 interacting with the residues of E612, S613 and E638 in Stat3.

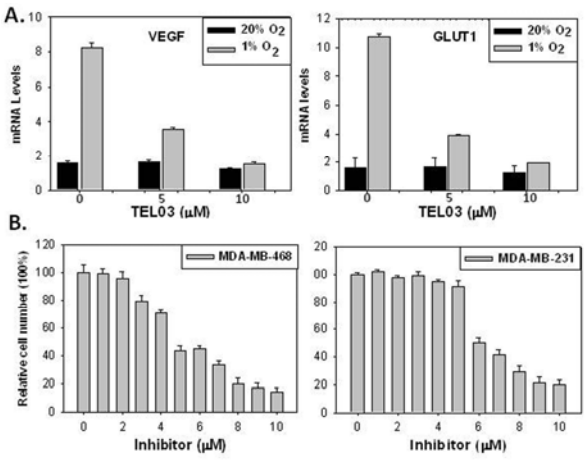

Fig.B9. (A) The mRNA levels of VEGF and GLUT1 was significantly suppressed by TEL03 in responding to hypoxia. (B) TEL03 induced apoptosis in MDA-MB-468 and MDA-MB-231 as well.

(4) TEL03 inhibits VEGF, GLUT1 and induces apoptosis. Expression of VEGF, which is a key stimulator of angiogenesis, and GLUT1, which can increase intracellular glucose uptake, are hypoxia-upregulated by HIF-1-dependent transcriptional activation (41). The results of quantitative RT-PCR showed that TEL03 significantly suppressed the expressions of VEGF and GLUT1 mRNA in responding to hypoxia (Fig.B9A). Also, MTT was employed to determine the activities of inducing cancer cell apoptosis for TEL03. The results showed that TEL03 significantly induced apoptosis in breast cancer cells (MDA-MB-468 and MDA-MB-231) (Fig.B9B).

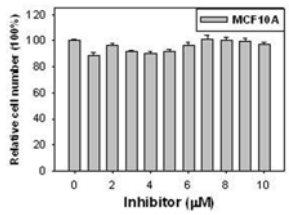

Fig.B10. MTT data from epithelial cells (MCF10A).

(5) Initial toxicity tests for TEL03. We also employed MTT to test whether TEL03 induces toxicity in normal breast cells (MCF10A). After adding TEL03 in breast epithelial cells (MCF10A) 48 hours, the data were collected under the same experimental

condition in breast cancer cells. TEL03 did not induce cell death in normal epithelial cells due to low expression of HIF-1α and p-Stat3 in epithelial cells, suggesting that TEL03 has a favorable safety profile (Fig.B10).

(6) Summary. Our results provided solid evidence that (1) in hypoxia TEL03 directly bound in the region of residues 796 to 801 of HIF-1α without disrupting HIF-1β and p300, blocked the interaction between p300 and residue N803 of HIF-1α and promoted the proteasomal degradation of HIF-1α, interrupted HIF-1α transcriptional activity, and greatly reduced the levels of hypoxia-regulated genes, including VEGF, GLUT1 and others (Fig.B11B). (2) TEL03 interacted with residues E612, S613 and E638 within the SH2 domain of Stat3 and disrupted Stat3 phosphorylation without interrupting the activations of p-Stat1 or p-Stat5, leading to decreased cancer cell proliferation, increased apoptosis, and reduced angiogenesis (Fig.B11A).

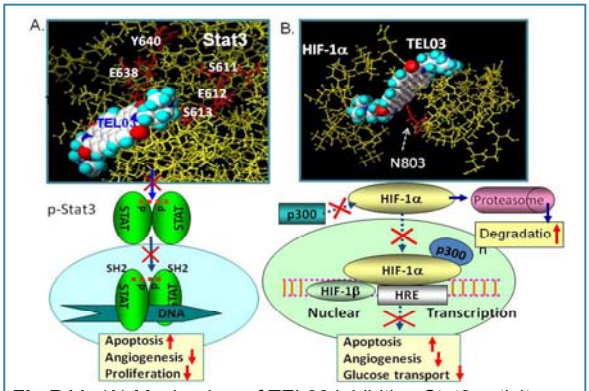

Fig.B11: (A) Mechanism of TEL03 inhibiting Stat3 activity. (B) Mechanism of TEL03 blocks HIF-1α transcription.

B6. TEL03 drug efficacy in prostate cancer

It is clear that a single prostate tumor may contain a group of cancer cells, and instead of the current "one-size-fits-all" treatment approach, a combined therapeutics is needed to target each cell group. HIF-1α and Stat3 are key factors that activates in all types of prostate cancers. TEL03 could be a potent candidate for future combination treatment in prostate cancer patients. Fig.B12 showed that TEL03 inhibited HIF1α/2α in PC3 and inhibited both HIF-1α/2α and p-Stat3 in DU145. We did not detect the expression of Stat3 and p-Stat3 in PC3 cells. Also, we performed in vivo assay to determine the drug efficacy of TEL03 using paclitaxel for comparison (Fig.13 & 14; Table). We gave the drug: TEL03 (2mg/kg) and paclitexal (10mg/kg) every other day for two weeks. The results demonstrated that TEL03 significantly suppressed PC3 tumor growth. The tumors in untreated mice gruw from 258 to 1582 mm^3; TEL03 suppressed the tumors from 245 to 235 mm^3 in two weeks. The results demonstrated that TEL03 significantly suppressed PC3 tumor growth.

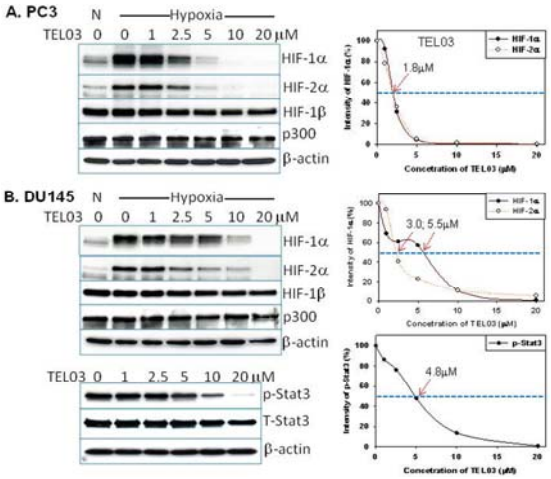

Fig.B12. (A) TEL03 inhibits HIF1α/2α in PC3 and IC50 =1.8μM; no Stat3 is detected in PC3. (B) TEL03 inhibits both HIF-1α/2α and p-Stat3 in DU145 and IC50s are 3~5μM.

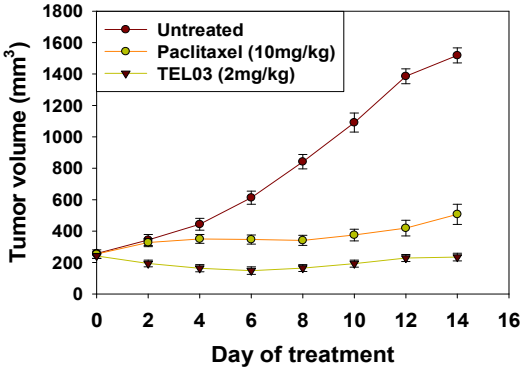

Fig.B13. (A) Plot of PC tumor volume vs. day of treatment.

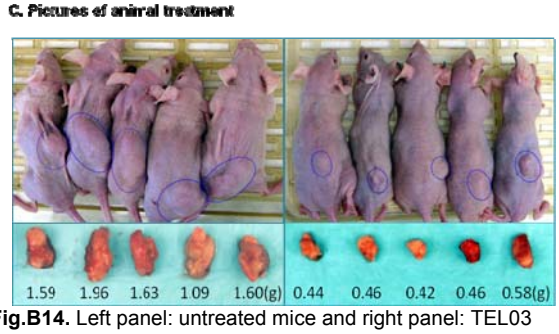

Fig.B14. Left panel: untreated mice and right panel: TEL03 (2mg/kg) treated mice

B .Table of *in vivo* results

Group	Drug dose	No. of mice Start	End	Weight of mice (g) Start	End	Tumor (mm^3) Start	End	Fold of tumor-growth	Mean-tumor weight (g)	P value
Control	untreated	5	5	27.0±0.6	25.5±1.8	258.0 ± 9	1582 ± 48	6.1	1.6 ± 0.1	-------
Paclitaxel	10mg/kg	5	5	29.7±0.7	27.5±1.3	254.4 ± 26	507 ± 64	2.0	1.0 ± 0.1	p<0.05
TEL03	2mg/kg	5	5	27.2±0.3	26.1±1.1	245.5 ± 15	235 ± 25	-0.1	0.5 ± 0.05	p<0.001

B7. *In vivo* drug efficacy in breast and pancreatic tumors

We have used the mice bearing breast (MDA-MB-231) and pancreatic (PANC1) tumors to inspect the ability of anti-cancer therapeutics of TEL03. After few days the tumors grew over 200mm^3, mice were randomly assigned to several groups (each group has seven mice) for drug treatments. We gave drugs to each mouse every other day for 10 days (6 treatments) (red arrows) through IP injection or oral route.

(1) The mice bearing breast tumors were treated in five groups: (i) non-treated; (ii) treated by a control agent TEL01 (2mg/kg), which shows no inhibition of both HIF-1α and Stat3 in Fig.B4 (#14); (iii) treated by T40214/PEI (10mg/kg), which is a Stat3 inhibitor delivered by PEI (2.5mg/kg) (34,35); (iv) treated by TEL03 (2mg/kg); and (v) oral treatment of TEL03 (5mg/kg). The results demonstrated (Fig.B14A) that without treatment the breast tumors grew aggressively to ~1860mm^3 in 15 days. TEL01 shows no drug activity. The growth of breast tumors was strongly suppressed by T40214 and TEL03 ($p<0.001$). The oral treatment of TEL03 also showed some drug activity ($p<0.02$). Comparing the treatment of T40214/PEI (10mg/kg+2.5mg/kg), a lower drug dose of TEL03 (2mg/kg) simply given to mice has strong drug efficacy *in vivo*. After the treatments tumors were harvested. The western blots from the obtained tumors showed (Fig.B15B) that TEL03 inhibited both HIF-1α and p-Stat3, and suppressed the expression of VEGF, but did not inhibit HIF-1β, p300 and T-State3, consistent with the observation in cells.

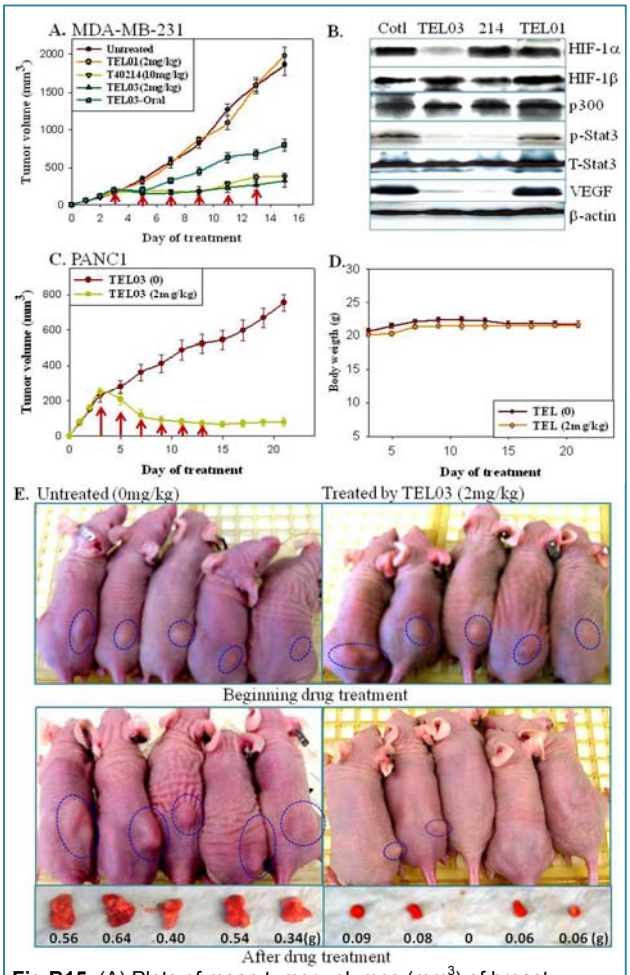

Fig.B15. (A) Plots of mean tumor volumes (mm^3) of breast tumors Vs days of drug treatment. (B) Western blots. (C) Plot of mean tumor volumes (mm^3) Vs days of drug treatment. (D) Plot of mean body weight (g) Vs days of drug treatment. (E) The pictures in left and right show the mice bearing tumors without treatment and with TEL03-treated, respectively.

(2) The mice bearing pancreatic tumors were treated in two groups: Group 1 was untreated; Group 2 was treated by TEL03 (2mg/kg) for 10 days and then monitored the tumor growth without treatment for another 9 days. Over 19 days the mean tumor volume in untreated mice increased from 225 to 754 mm^3; in contrast, the mean tumor volume in TEL03-treated mice was decreased from 246 to 75 mm^3 (Fig.B15C). At the endpoint, the mean tumor weight of untreated mice was 0.5 ± 0.05g whereas the mean tumor weight of TEL03-treated mice was 0.06 ± 0.01g (Fig.B14E). The results showed that after given with 2mg/kg of TEL03 in 6 times, the mean tumor volume of pancreatic tumors was dramatically suppressed and the tumors did not re-grow after stopping treatment in 9 days. The body weight of the treated mice was not affected by TEL03 treatment (Fig.B15D). Together, the agent TEL03 demonstrated greater-than-expected *in vivo* potency since this inhibitor has the ability to promote drug efficacy, reduce emergence of therapy-

resistant cancer cells, and decrease the incidence of cancer relapse without introducing additional toxic side effects. Thus, targeting both HIF-1α and Stat3 is considered as a promising strategy for breast cancer therapy.

C. CONCLUSION

Our main goal is to develop novel therapeutic agents from Chinese herbal medicine (CHM) that targets HIF-1α /2α for prostate cancer therapy. Hypoxia orchestrated by HIF-1α is crucial for tumor progression, therapy resistance, and poor patient outcome. Also, HIF-1α regulates several critical pathways in human cancers, including PI3K, AKT and MEK/ERK pathways. Overexpression of HIF-1α in breast tumors not only strongly enhances the tumor growth rate and metastatic potential, but also contributes to resistance to radiotherapy and chemotherapy, and is associated with treatment failure and increased patient mortality. Stat3 participates in oncogenesis through the upregulation of genes encoding anti-apoptosis, cell-cycle regulators, and inducers of angiogenesis in many cancers, inclosing breast cancer. Also, Stat3 signaling is a major intrinsic pathway of cancer inflammation and mediates the cancer-promoting properties. Stat3 suppresses anti-tumor immune responses and promotes inflammation-induced cancer, making it as a critical target for cancer therapy.

Recently, we have identified a perylene derivative TEL03 from *hypocrellin* (CHM: *Hypocrella bambusae*) that significantly inhibits activations of HIF-1α/2α and p-Stat3, and blocks the expression of their down-regulated oncogenes (e.g. Bcl2, VEGF, Glut1, and others) in cancer cells. TEL03 also dramatically suppressed the growth of prostate, breast and pancreatic tumors in xenograft models. Our previous studies provided evidence that targeting both HIF-1α and Stat3 together could improve tumor response to either agent alone, and reduce drug resistance and treatment failure. TEL03 was showed inhibition of tumor growth and a marked delay in tumor re-growth, and demonstrated a greater-than-expected *in vivo* potency. Our results also provided evidence that TEL03 has potential to be a potent anti-cancer agent for pancreatic cancer therapy, suggesting that TEL03 could have a possible clinical application in prostate cancer therapy.

D. OVERALL PROJECT SUMMARY

The title of this idea development project is: Development of a combination therapy for prostate cancer by targeting Stat3 and HIF-1alpha, which was founded for three years (7/2010-6/2013).

In this three-year period, **(1)** we have originally proposed the hypothesize that activation of Stat3 and HIF-1 strongly influence progression of prostate cancer, using a strategy that targets both Stat3 and HIF-1α could produce an effective treatment for prostate cancer. Our systemic results demonstrated (28) that a combination treatment of T40214 (a Stat3 inhibitor) and JG244 (HIF-1α inhibitor) to target both p-Stat3 and HIF-1α together significantly induces cancer cell apoptosis and greatly enhances in vivo drug efficacy as compared with single agent that blocks activation of either p-Stat3 or HIF-1α molecule alone. The combination treatment including a HIF-1α/2α inhibitor (JG244) not only has therapeutic efficacy in targeting HIF-1α/2α, but also could reduce the hypoxia-induced drug resistance to other therapies and enhance drug efficacy. **Our studies demonstrated that (i) the combination treatment by targeting both Stat3 and HIF-1α together could make prostate cancer treatments more effective; and (ii) a combination treatment including a HIF-1α inhibitor could be a pioneer strategy for prostate cancer therapy.**

(2) Based on our first studies, we have developed TEL03, a dual-inhibitor that targets both HIF-1α/2α and p-Stat3 for treatment of prostate and other cancers. TEL03, which is a novel anti-cancer agent derived from Chinese herbal medicine (CHM: *Hypocrella bambusae*), can promote drug efficacy, reduce emergence of therapy-resistant cancer cells, and decrease the incidence of cancer relapse with a low toxicity. CHM focuses on restoring a balance of energy, body, and spirit to maintain health rather than treating a particular diseases or medical condition. Also, they can help ease the side effects of

standard cancer treatment, control pain, improve quality of life, strengthen the immune system, and in some cases, stop tumor growth and spread. Therefore, TEL03 has a potential to be a novel clinical application in prostate cancer therapy. **These studies provided some critical information: (i) a dual inhibitor that targets both HIF-1α and Stat3 simultaneously could be improve tumor response, reduce drug resistance and treatment failure; and (ii) anti-cancer agents obtained from CHM could have promising drug efficacy for prostate cancer therapies.**

(3) There still have more works need to do for developing a potent therapeutic agent for prostate cancer therapy when three years passed, such as: (I) to optimize TEL03 in order to make the agent more efficiency by performing medicinal chemistry and QSAR studies; (II) to test more in vivo studies, including different prostate cancer tumors, such as DU145 tumor that highly resists clinical drug treatment, and perform the cooperated *in vivo* efficacy in combination treatment using clinic drugs (e.g. docetaxel and others) with TEL03 to determine whether TEL03 has a great potential for clinical usage; and (III) screen more new compounds from CHM to discover a high potent inhibitor.

E. OVERALL PROJECT OUT COMES

Publications relevant to the project in this period:

1. Reddy KR, Guan Y, Qin G, Zhou Z, Jing N. "Combined treatment targeting HIF-1α and Stat3 is a potent strategy for prostate cancer therapy" *Prostate* (2011) 16:1796-809.
2. Jing N. "Rational design of DNA anti-cancer agent that target signal transducer and activator of transcription (Stat3) for cancer therapy" *Book: CELL SIGNALLING and MOLECULAR TARGETS IN CANCER*. Springer Science LLC 2012:167-190.
3. Chen H, Guan Y, Yuan G, Tweardy DJ, Jing N. "A perylene derivative TEL03 targets HIF-1α and Stat3 for cancer therapy" *In press* (2013).

Patent application (collaboration between China and Jing).

1. Chinese patent (pending, 2012): Perking University, Jing N (BCM), Guan Y (BCM). "二萘嵌苯衍生物作为p-STAT3/Hif1α信号传导通路抑制剂在相关疾病中的应用" (application of perylene derivatives that inhibit p-Stat3/HIF-1α in medical diseases) (the contributions of Baylor College of Medicine (BCM), USA, and Perking University, China, are 50:50).

Abstracts presented in conferences.

1. Reddy KR, Guan Y, Jing N. Combination of targeting HIF-1α and Stat3 is a potent strategy for prostate cancer therapy. AACR-NCI-EORT, November, 2010.
2. Chen H, Guan Y, Yuan G, Tweardy DJ, Jing N. TEL is a novel dual anti-cancer agent that targets Stat3 and HIF-1α. AACR-NCI-EORT, November 2011.

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10. Wang, G. L. and Semenza, G. L. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem, *270:* 1230-1237, 1995.
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GENERAL BIOGRAPHICAL INFORMATION

A. Personal
Name: **Naijie Jing**

B. Education:

a. Undergraduate Education:
Huaiyin Normal University, Jiansu Province, China, B.S, 1981

b. Medical Education or Graduate Education
The University of Alabama at Birmingham, Alabama, M.S. (Biophysics), 1988
The University of Alabama at Birmingham, Alabama, Ph.D. (Biophysics), 1994

c. Postgraduate Training:
Postdoctoral Fellow, Laboratory of Molecular Biophysics, School of Medicine, University of Alabama at Birmingham, Birmingham, AL, Mentor: Dan W. Urry, 1994 – 1995

Postdoctoral Associate, Department of Molecular Physiology and Biophysics, Baylor College of Medicine Houston, TX, Mentor: Michael E. Hogan, 1995 – 1998

C. Academic Appointments:

a. Current Faculty Position:
Associate Professor, Section of Infectious Diseases, Department of Medicine
Baylor College of Medicine, Houston, Texas, 2005 – present.

Faculty members in Cancer Center, AIDS Center and Keck Center of Computational and Structural Biology
Baylor College of Medicine, Houston Texas, 2001-present

b. Previous faculty position at other institutions:
Assistant Professor, Section of Infectious Diseases, Department of Medicine
Baylor College of Medicine, Houston, Texas, 2001 – 2005.

Assistant Professor, Department of Molecular Physiology and Biophysics
Baylor College of Medicine, Houston, Texas 1999 – 2001

Instructor, Department of molecular Physiology and Biophysics
Baylor College of Medicine, Houston, Texas 1998 – 1999

II. RESEARCH INFORMATION

A. Research Supports

Funded Awarded

a. Title: Development of a combination therapy for prostate cancer by targeting Stat3 and HIF-1α.
b. Funding Agency: Department of Defense/ US Army, PC093258.
c. PI: Naijie Jing
d. Dates of Funding: 7/1/20010 -6/30/2013.
e. Grant or Contract: DOD Grant.

a. Title: A novel inhibitor of Stat3 for prostate cancer therapy
b. Funding Agency: National Institute of Health/NCI, CA104035.
c. PI: Naijie Jing
d. Dates of Funding: 4/1/2005 – 2/28/2011
e. Grant or Contract: NIH R01Grant

a. Title: GQ-ODN T40214: A novel and potent anti-cancer agent for prostate cancer therapy
b. Funding Agency: Prostate Cancer Foundation
c. PI: Naijie Jing
d. Dates of Funding: 3/1/2007 –2/28/2008

e. Grant or Contract: PCF Competitive Award.

a. Title: A novel strategy of chemotherapy for head and neck with GQ-ODN, a novel anti-cancer agent.
b. Funding Agency: NIH/NCI/SPORE of Head and Neck in MD Anderson, CA097007.
c. Project PI: Naijie Jing
d. Dates of Funding: 3/1/2005 – 2/28/2007
f. Grant or Contract: NIH Development Grant

a. Title: A novel strategy of chemotherapy for head and neck with GQ-ODN, a novel anti-cancer agent.
b. Funding Agency: NIH/NCI/SPORE of prostate cancer in Baylor, CA058204.
c. Project PI: Naijie Jing
d. Total Dates of Funding: 9/1/2005 –8/31/2007
e. Grant or Contract: NIH Development Grant

a. Title: Targeting Stat3 with G-quartet oligonucleotides in metastatic prostate cancer
b. Funding Agency: Department of Defense/ US Army, PC020407.
c. PI: Naijie Jing
d. Dates of Funding: 4/1/2003 -3/31/2006
e. Grant or Contract: DOD Grant.

a. Title: Developing a potent inhibitor of HIV-1 integrase
b. Funding Agency: NIH/GM, GM 60153
c. PI: Naijie Jing
d. Dates of Funding: 9/1/1999 – 8/31/2003
e. Grant or Contract: NIH R01Grant.

B. National and International Scientific Participation:

a. Journal editorial boards:
Editorial Board, *Current Molecular Pharmacology*.
Guest Editor, *Current Pharmaceutical Design.*
Editor Board, *Recent Patent Reviews on Anti -Cancer Drug Discovery*
Editorial Board, *Clinical Pharmacology: Advances and Applications.*
b. Professional societies:
Overseas Fellow of Royal Society of Medicine (London, England)
Member of American Chemical Society (ACS)
Member of American Association for the Advancement of Science (AAAS)
Member of New York Academy of Science
Member of American Associate of Cancer Research (AACR)
c. Study section.
ZRG1 OTC-X (02) Translational Clinical Oncology, NCI/NIH, 2011
ZRG1 OTC-X (90) Cancer Therapeutics AREA Grant Applications Study Section, 2012

C. Patents approved and issued:

I. US patents:

1. Jing, N. et al., "A Novel Technology of Intracellular Delivery of DNA Oligonucleotides to Improve Drug Activity" USPTO Application#:2006199777.

2. Jing N and Guan Y "G-Quartet oligonucleotides that target hypoxia-induced factor 1-α (HIF1α)" USPTO Application #: 20090075928.

II. Chinese patent (pending, 2012):

1. Yuan G, **Jing N**, Chen H, Guan Y. "二萘嵌苯衍生物作为 p-STAT3/Hif1α 信号传导通路抑制剂在相关疾病中的应用"(application of perylene derivatives that inhibit p-Stat3/HIF-1α in medical deseases) (the contributions of Baylor college of Medicine, USA, and Perking University, China, are 50:50).

D. Publications:

a. Full papers (published in referred journals):

1. Dan W. Urry, **Naijie Jing**, and K. U. Prasad, "On the Mechanism of Channel-Length Dependence of Gramicidin Single-Channel Conductance", (1987) *Biochimica et Biophysica Acta* 902:137-144.

2. Dan W. Urry, **Naijie Jing**, Tina L. Trapane, Chi-Hao Luan, and Marsh Waller, "Ion Interaction with the Gramicidin A Transmembrane Channel: Cesium-133 and Calcium-43 NMR Studies", (1988) *Current Topics in Membrane and Transport* 33:51-90.
3. Dan W. Urry, D. Channe Gowda, Shaoqing Peng, Timoty M. Parker, **Naijie Jing**, and R. Dean Harris, "Monometric Design of Extraordinary Hydrophobicity-induced pKa Shifts for Aspartic Acid: Relevance to Protein Mechanisms", (1994) *Biopolymers* 34:889-896.
4. **Naijie Jing**, Kari U. Prasad, and Dan W. Urry, "NMR Determination of Conformation of Ion-Transport-Competent Gramicidin in DPC Micelles: A Right-Handed, Head-to-Head Dimerized, Single-Stranded $\beta^{6.3}$-Helix", (1994) *Biophysical J.* 66(2): A353.
5. Dan W. Urry, D. T. McPherson, J. Xu, H. Daniell, C. Guda, D. C. Gowda, **Naijie Jing**, T. M. Parker, "Protein-Based Polymeric Material: Syntheses and Properties" (1995) in *The Polymeric Materials Encyclopedia: Sythesis, Properties and Applications*, CRC Press, Boca Raton, pp. 2645-2699.
6. **Naijie Jing**, Kari U. Prasad, and Dan W. Urry, "The Determination of Binding Constants of Micellar Packaged Gramicidin A by ^{13}C and ^{23}Na NMR", (1995) *Biochimica et Biophysica Acta* 1238: 1-11.
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8. D. G. Gowda, C. H. Luan, R. L. Furner, S. Q. Peng, **N. Jing**, C. M. Harris, T. M. Parker, and D. W. Urr "Synthesis and Characterization of the Human Elastin W4 Sequence", (1995) *International Journal of Peptide & Protein Research* 46:453-463, 1995.
9. Dan W. Urry, Larry C. Hayes, D. Channe Gowda, Shao Qing Peng and **Naijie Jing**, "Electro-Chemical Transduction in Elastic Protein-Based Polymers: A Model for an Energy Conversion Step of Oxidative Phosphorylation" (1995) *Biochemical Biophysical Research Communication* 210(3): 1031-1039.
10. Dan W. Urry, Asima Pattanaik, Mary Ann Accavitti, Chi-Xiang Laun, David T. McPherson, Jie Xu, D. Channe Gowda, Timothy M. Parker, Cynthia M. Harris, and **Naijie Jing** "Transductional Elastic and Plastic Protein-Based Polymers as Potential Medical Devices" (1996) in *Handbook of Biodegradable Polymers* (eds. Domb, Kost, and Wiseman) by Harwood Academic Publishers, Chur, Switzerland, 2412-2429.
11. **Naijie Jing**, Robert F. Rando, Yves Pommier, and Michael E. Hogan "Ion Selective Folding of Loop Domains in a Potent Anti-HIV Oligonucleotide" *Biochemistry* (1997) 36: 12498-12505.
12. **Naijie Jing**, Xiaolian Gao, Robert F. Rando, and Michael E. Hogan "Potassium-Induced loop Conformational Transition of a Potent Anti-HIV Oligonucleotide" *Journal of Biomolecular Structure and Dynamics* (1997) Vol.15(3): 573-585.
13. **Naijie Jing** and Michael E. Hogan "Structure-Activity of Tetrad-forming Oligonucleotide as a Potent Anti-HIV Therapeutic Drug" *The Journal of Biological Chemistry* (1998) 273 (52): 34992-34999.
14. **Naijie Jing**, De, Clercq, E., Rando, R. F., Pallansch, L., lackman-Smith, C., Lee, S., and Hogan, M. E. "Stability-Activity Relationships of a Family of G-tetrad forming Oligonucleotides as Potent HIV Inhibitors: A Basis for Anti-HIV Drug Design" *The Journal of Biological Chemistry* (2000) 275 (5): 3421-3430.
15. **Naijie Jing**, Marchand, C., Liu, J., Mitra, R., Hogan, M. E., and Pommier, Y. "Mechanism of Inhibition of HIV-1 Integrase by G-tetrad Forming Oligonucleotides *in Vitro" Journal of Biological Chemistry* (2000) 275 (28), 21460-21467.
16. **Naijie Jing** "Developing G-quartet oligonucleotides as novel anti-HIV agents: focus on anti-HIV drug design" *Expert Opinion on Investigational Drugs* (2000) 9(8) 1777-1785.
17. **Naijie Jing**, Xuejun Xu "Rational Drug Design for DNA Oligonucleotides as Potent HIV Inhibitors" *Current Drug Targets-Infectious Disorder* (2001) 1(2), 79-90.
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4. **Naijie Jing***, Li, Y., Xiong, W., Sha, W. Jing, L. Tweardy, D. J. "G-quartet oligonucleotides: a new class of Stat3 inhibitors that suppresses growth of prostate and breast tumors through induction of apoptosis" 95th AACR conference, 2004.
5. **Naijie Jing***, Yidong Li, Wei Sha, Weijun Xiong, David J. Tweardy "A novel strategy to inhibit Stat3 signaling for human cancer therapy" Molecular Target and Cancer Therapeutic, EORTC-NCI-AACR meeting, 2004.
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7. **Naijie Jing***, Yidong Li, Qiging Zhu, Ping Yuan, Yun Oh, Li Mao and David J. Tweardy "Suppression of growth of squamous cell carcinoma of head and neck xenografts in nude mice by G-quartet oligonucleotides that target Stat3" 96th AACR conference April 16-21, 2005, Anaheim CA.
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14. **Jing** et al. A Novel Strategy for Prostate Cancers Therapy by Targeting Stat3 with G-quartet Oligonucleotides. 14th Prostate Cancer Foundation Scientific Retreat Oct, Lake Tahoe, NE 11-13, 2007.
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